Pestic. Sci. 1998, **53**, 311–323

Fate of Metsulfuron-methyl in Soils in Relation to Pedo-climatic Conditions

Nelly Pons[1,2] & Enrique Barriuso[2]*

[1] ITCF, 91720 Boigneville, France
[2] Unité de Science du Sol, INRA, 78850 Thiverval-Grignon, France

(Received 12 December 1997; accepted 6 April 1998)

Abstract: The dependence of the behaviour of metsulfuron-methyl on soil pH was confirmed during incubations under controlled laboratory conditions with two French soils used for wheat cropping. The fate of [^{14}C] residues from [*triazine*-^{14}C]metsulfuron-methyl was studied by combining different experimental conditions: soil pH (8·1 and 5·2), temperature (28 and 10°C), soil moisture (90 and 50% of soil water holding capacity) and microbial activity (sterile and non-sterile conditions). Metsulfuron-methyl degradation was mainly influenced by soil pH and temperature. The metsulfuron-methyl half-life varied from five days in the acidic soil to 69 days in the alkaline soil. Under sterile conditions, the half-life increased in alkaline soil to 139 days but was not changed in the acidic soil. Metsulfuron-methyl degradation mainly resulted in the formation of the amino-triazine. In the acidic soil, degradation was characterised by rapid hydrolysis giving two specific unidentified metabolites, not detected during incubations in the alkaline soil. Bound residues formation and metsulfuron-methyl mineralisation were highly correlated. The extent of bound residue formation increased when soil water content decreased and was maximal [48 (±4)% of the applied metsulfuron-methyl after 98 incubation days] in the acidic soil at 50% of the water holding capacity and 28°C. Otherwise, bound residues represented between 13 and 32% of the initial radioactivity. © 1998 SCI

Pestic. Sci., **53**, 311–323 (1998)

Key words: sulfonylurea; availability; biological degradation; abiotic degradation; soil moisture; soil temperature; pH

1 INTRODUCTION

Sulfonylurea herbicides have high activity at very low application rates. Among sulfonylureas, metsulfuron-methyl is widely used, with good selectivity, against a wide range of weeds in cereal crops at application rates of 4 to 8 g AI ha^{-1}.[1,2] The efficiency of this sulfonylurea is related to its fate in soils, and its bioavailability depends on its degradation and its retention on soil constituents. Most pesticides are degraded in soils mainly through biological processes involving soil micro-organisms. However, the relative importance of abiotic versus biotic degradation often remains unknown. Physicochemical factors as soil pH, soil water content and temperature can influence both biological and abiotic degradation processes.[3–10] Abiotic degradation of sulfonylurea herbicides has been demonstrated and can become predominant in acidic conditions. The dependence of metsulfuron-methyl persistence in soils on soil pH is well documented.[7–12] However, there is a lack of knowledge about the extent of the influence of physicochemical factors on both abiotic and biological degradation process.

The fate of sulfonylurea in soils is directly related to their chemical structure and mainly to the ionisation of

* To whom correspondence should be addressed.
Contract/grant sponsor: French Minister of Agriculture.

 Pestic. Sci. 0031-613X/98/$17.50. Printed in Great Britain

the sulfonylurea bridge. They are weak acids with pKa from 3 to 5 (3·3 for metsulfuron-methyl) and in soils they are mainly in the ionised form. This explains their low sorption coefficients, which are pH-dependent.[2,8,11] Their abiotic degradation also varies with pH in soil, decreasing when the soil pH increases.[13–15] This has been explained by the lower sensitivity to abiotic hydrolysis of the ionic forms compared to the non-ionic forms.[16] However, this explanation was recently contested by a theoretical calculation of the atomic charges at the carbonyl-carbon, which indicated that the ionic species are more susceptible towards hydrolysis by a nucleophilic attack than the neutral species and that faster reactions at lower pH may be due merely to the higher proton concentration.[15]

The dependence of the degradation rate on pH has been used to establish guidelines governing the use of the more persistent sulfonylureas.[2] Rapid degradation in acidic conditions can be detrimental to efficient plant protection. On the other hand, low degradation in alkaline soil where abiotic degradation is minimised might induce damage in rotational cropping. Indeed, soil residues of metsulfuron-methyl have been reported to cause some damage to rotation or substitution crops.[17,18]

Although the soil persistence of many sulfonylureas is well documented, few data are available about the behaviour of the transformation products in soil and very few global balances of the residues of sulfonylurea, particularly metsulfuron-methyl, have been published.[19,20] This work was initiated to obtain results about the fate of metsulfuron-methyl in typical French soils used for wheat cropping. The main objectives were to identify the relative importance of the abiotic and biological processes of metsulfuron-methyl degradation, and to determine the influence of physicochemical parameters (soil pH, temperature and humidity) and soil microbial activity (sterile and non-sterile conditions) on these. The studies were run in controlled laboratory conditions using [^{14}C]-labelled herbicide, from which a mass balance of metsulfuron-methyl residues could be obtained. Special attention was given to the detection of degradation products in order to relate the metabolic pathway to the experimental conditions. Most of the metabolites remained unidentified; however their variety and relative proportions were informative and could stimulate complementary studies and more extensive metabolic identification.

2 MATERIALS AND METHODS

2.1 Chemical

Metsulfuron-methyl [methyl 2-(4-methoxy-6-methyl-1,3,5-triazin-2-ylcarbamoylsulfamoyl)benzoate [^{14}C]-labelled on the first carbon of the triazine ring, was supplied by Du Pont de Nemours. Its specific activity was 1·3 MBq mg^{-1}, and the radiopurity was greater than 98%. Water holding capacity of the soil was adjusted with the aqueous solution of [^{14}C]metsulfuron-methyl applied on the soil. Two solutions at 0·09 and 0·27 mg litre^{-1} ($4{\cdot}3 \times 10^8$ and $1{\cdot}1 \times 10^9$ Bq litre^{-1}) were prepared by dissolving [^{14}C]metsulfuron-methyl in water.

2.2 Soils

Two soils were used: a silty clay loam soil sampled in Boigneville (Bg) (France) and a sandy loam soil from Vrigny (Vg) (France). Both soils came from plots cultivated with wheat. They mainly differed in their pH in water: 8·1 for Bg soil and 5·2 for Vg soil. Soils were sampled in the upper 0–20 cm and sieved at 2 mm. The main characteristics of both soils are shown in Table 1.

2.3 Incubation experiments

Several incubations were carried out in parallel to test different factors: soil type (Bg and Vg), temperature (10 and 28°C), soil water content (50 and 90% of the water holding capacity, WHC), and sterile and non-sterile conditions. Experimental design was done to combine these factors, as shown in Table 2. For each incubation, 40 g of fresh soil treated with 23 μg of [^{14}C]metsulfuron-methyl kg^{-1} dry soil (corresponding to 30 kBq kg^{-1}) were introduced in sealed 500-ml jars. The [^{14}C]metsulfuron-methyl solution in water was used to adjust the soil water content, complemented with water when necessary. Jars were placed in dark rooms with controlled temperature: 10 (±2) and 28 (±1)°C. Incubations in sterile conditions were done with soils previously sterilised by autoclaving three successive times of 30 min at 120°C.

In each jar a vial containing sodium hydroxide solution (1 M; 5 ml) was introduced to trap the [^{14}C]carbon dioxide evolved. These vials were changed periodically,

TABLE 1
Main Characteristics of Boigneville (Bg) and Vrigny (Vg) Soils

Soil	*Clay*	*Silt*	*Sand*	$CaCO_3$	*pH* (*water*)	*C*	*N*	*Water holding capacity* (*g water kg*$^{-1}$)
	(*g kg*$^{-1}$)					(*g kg*$^{-1}$)		
Bg	308	578	114	67	8·1	12·9	1·5	343
Vg	98	310	592	<1	5·2	8·7	1·0	332

TABLE 2
Experimental Design of [^{14}C]Metsulfuron-methyl Incubations Crossing Soil Type (Boigneville, Bg and Vrigny, Vg), Sterile/Non-sterile Conditions, Soil Water Content and Temperature

	Soil		*Sterile soil*		*Water content (%)*[a]		*Temperature (°C)*		
No.	*Bg*	*Vg*	*Bg*	*Vg*	*50*	*90*	*10*	*28*	*Notation*
1	X					X		X	Bg90/28
2		X				X		X	Vg90/28
3	X					X	X		Bg90/10
4		X				X	X		Vg90/10
5	X				X			X	Bg50/28
6		X			X			X	Vg50/28
7	X				X		X		Bg50/10
8		X			X		X		Vg50/10
9			X			X		X	St-Bg90/28
10				X		X		X	St-Vg90/28
11			X		X			X	St-Bg50/28
12				X	X			X	St-Vg50/28

[a] Of the total water holding capacity.

and soil water content was adjusted monthly by weighing. Soil samples were recovered after 14, 42, 70 and 98 days of incubation. Separate jars were prepared for each sampling date, and all samples were done in triplicate.

2.4 Analysis

The radioactivity in the sodium hydroxide traps was measured periodically by liquid scintillation counting with a Kontron Betamatic V counter (Kontron Ins., Montigny le Bretonneux, France) using Picofluor (Packard) as scintillation liquid.

After 14, 42, 70 and 98 days of incubation, the residual [^{14}C]metsulfuron-methyl was extracted from the soils with aqueous calcium chloride (0·01 M; 2 × 50 ml), then with methanol + water + acetic acid (8 + 1·9 + 0·1 by volume; 2 × 50 ml). For each extraction, soil samples were shaken during 24 h, then the extracts were recovered after centrifugation (10 000*g* for 10 min). The radioactivity content of the extracts was measured by liquid scintillation counting. The non-extractable radioactivity, corresponding to the 'bound residues', was measured by scintillation counting of the [^{14}C]carbon dioxide evolved after combustion of the unextractable solid residues (Sample Oxidizer 307, Packard, Meriden, CT, USA).

HPLC analyses were only carried out on aqueous extracts because the radioactivity content of the methanol extracts was too low. Aqueous extracts were concentrated by solid/liquid extraction with cartridges of 200 mg of polystyrene divinylbenzene (Lichrolut EN; Merck) previously activated by passing methanol (5 ml) and then water (5 ml). Water extracts were passed through the activated cartridges under a slight vacuum, and the cartridges were then dried by passing air through them for 15 min. The radioactive residues were then eluted with methanol (20 ml), then concentrated until dryness by evaporation under vacuum at 40°C with a Rotavapor (Büchi RE 111). Residues were dissolved in 2 ml of the initial solvent used for the HPLC analyses, filtered through a Cameo 13N syringe nylon filter (0·45 μm; MSI, Westboro, MA, USA). Samples were analysed using a Waters HPLC appliance (600E Multisolvent Delivery System, 717 Autosampler and a Novapak C18 column; 5 μm, 250 × 3·5 mm; Waters, Milford, MA, USA) equipped with a Waters 996 photodiode array detector coupled on-line with a radioactive flow detector (Packard-Radiomatic Flo-one A-550). The mobile phase was methanol + water with 0·01 M tetrabutylammonium chloride, starting at 20 + 80 (by volume) and reaching 90 + 10 (by volume) after 20 min with a linear gradient; the last solvent was then kept for 15 min. The mobile phase flow was 0·7 ml min^{-1}, and the injected sample volume 800 μl. Chromatographic peaks were characterised by their retention time compared to those of the standard products. Only metsulfuron-methyl and two pure metabolites [methyl 2-(4-hydroxy-6-methyl-1,3,5-triazine-2-yl carbamoylsulfamoyl) benzoate and 4-methyl-6-methoxy-2-amino-1,3,5-triazine, referred to as hydroxy-metsulfuron-methyl and amino-triazine respectively] were available for identification; others peaks were designated by their retention times (NI—non identified–plus retention time in min).

3 RESULTS AND DISCUSSION

3.1 Behaviour of [^{14}C]metsulfuron-methyl in relation to soil type

The distribution of radioactivity from [^{14}C]metsulfuron-methyl among the different compartments analysed during soil incubations at a moisture content of 90% WHC and 28°C is summarised in Fig. 1. These conditions are assumed to be optimal for the biological degradation of most pesticides in soils. Under these conditions, the fate of [^{14}C]metsulfuron-methyl was very dependent on the soil type. In the alkaline Bg soil, [^{14}C]metsulfuron-methyl was degraded more slowly than in the acidic Vg soil. It is well known that sulfonylurea degradation in soil depends on pH because abiotic hydrolysis of the sulfonylurea bridge is favoured at low pH.[15,16] After 98 days of incubation, 8% of the metsulfuron-methyl remained in the water extracts from the Bg soil, but it had totally disappeared after 42 days in the Vg soil. In this soil, the metsulfuron-methyl was degraded both by micro-organisms and by abiotic hydrolysis. The kinetics of metsulfuron-methyl degradation could be described using a first-order equation. The corresponding degradation rates were: 0·041 (±0·005) and 0·140 (±0·001) days^{-1} in Bg and Vg soils respectively, corresponding to half-lives of 17 and five days respectively (Table 3).

During soil incubation, the nature of the degradation products and the kinetics of their formation differed in the two soils (Fig. 1). Figure 2 shows examples of chromatograms representing the metabolic profiles of the metsufuron-methyl in Bg and Vg soils incubated at 90% WHC and 28°C. In the alkaline Bg soil, the metabolite detected was mainly the amino-triazine, which represented up to 38% of the applied metsulfuron-methyl between 40 and 70 days of incubation. The second metabolite detected was hydroxy-metsulfuron-methyl, which was present at all sampling times, and, at the end of the incubation, represented 9% of the applied metsulfuron-methyl. During the last days of incubation another non-identified metabolite (NI-25) appeared with a chromatographic retention time of 25 min (Fig. 1). In the acidic Vg soil, additional non-identified metabolites were detected (Figs 1 and 2): NI-18, NI-15 and NI-5, which were present only as traces in Bg soil. In the Vg soil, hydroxy-metsulfuron appeared after 14 days of incubation, but its proportion decreased during the incubation and was very low at the end. The proportion of amino-triazine was lower than in the Bg soil, and represented between 12 and 21% of the applied metsulfuron-methyl. The non-identified metabolite NI-18 was detected rapidly and represented between 11 and 15% of the applied metsulfuron-methyl. This metabolite seemed specific to metsulfuron-methyl degradation in this acidic soil. Other minor transitory non-identified metabolites (NI-15 and NI-5) were detected during the incubations. The NI-25 detected in Bg soil was never present in Vg soil incubated at 28°C (Figs 1 and 2).

Amino-triazine and hydroxy-metsulfuron-methyl characterise the two main metabolic pathways of degradation of metsulfuron-methyl: hydrolysis of the sulfonylurea bridge and *O*-demethylation of the methoxy-triazine moiety.[14,21–23] Classically it was considered that hydrolysis of the sulfonylurea bridge was the main mechanism of sulfonylurea degradation.[2,8] However, *O*-demethylation was confirmed as another

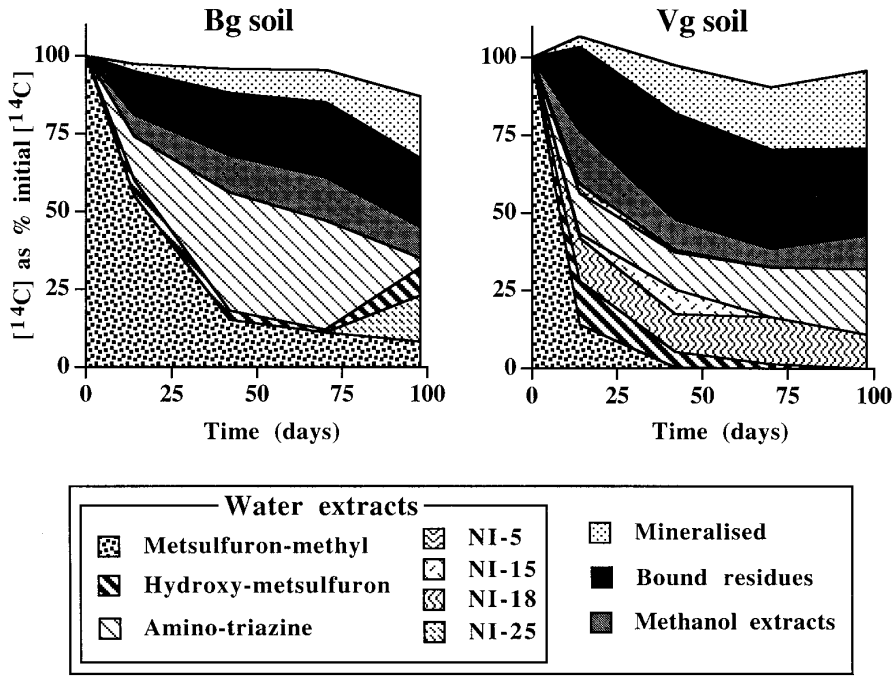

Fig. 1. Fate of [^{14}C]metsulfuron-methyl during incubations at 90% WHC and 28°C in alkaline Bg and acidic Vg soils.

TABLE 3

Distribution at the End of the Different Incubations of the Radioactivity of the Applied [^{14}C]Metsulfuron-methyl between the Fraction Mineralised, the Water (0·01 M $CaCl_2$) and Methanol (Methanol + Water + Acetic + Acid: 8 + 1·9 + 0·1) Extracts and the Bound Residues

Incubation[a]	*Mineralised*	*Water extracts*	*Methanol extracts*	*Bound residues*	*Half-life*[b] (*days*)
	(% *of initial* [^{14}C])				
Bg90/28	19·8 (±0·5)	42·1 (±2·9)	9·8 (±0·5)	22·1 (±2·4)	17 (±2)
Vg90/28	25·1 (±0·7)	31·3 (±0·4)	6·2 (±0·2)	28·1 (±6·6)	5 (±0)
Bg90/10	2·6 (±0·1)	66·0 (±5·1)	9·3 (±0·8)	14·6 (±1·1)	53 (±10)
Vg90/10	4·9 (±0·3)	53·8 (±2·4)	25·1 (±0·4)	13·7 (±2·2)	17 (±3)
Bg50/28	14·2 (±0·1)	43·3 (±0·7)	10·7 (±0·5)	31·7 (±0·2)	22 (±3)
Vg50/28	29·1 (±1·7)	22·1 (±0·3)	3·4 (±0·5)	48·1 (±4·1)	5 (±0)
Bg50/10	2·8 (±0·2)	68·5 (±1·6)	9·9 (±2·0)	11·6 (±2·2)	69 (±8)
Vg50/10	5·0 (±0·1)	45·8 (±2·1)	24·6 (±0·8)	19·2 (±0·6)	17 (±4)
St-Bg90/28	0·3 (±0·0)	65·1 (±4·3)	11·0 (±0·4)	17·1 (±0·3)	139 (±39)
St-Vg90/28	0·1 (±0·0)	70·0 (±2·4)	1·0 (±0·7)	29·0 (±4·6)	5 (±0)
St-Bg50/28	0·1 (±0·1)	78·5 (±2·0)	9·8 (±0·1)	11·4 (±0·3)	99 (±12)
St-Vg50/28	0·1 (±0·0)	52·1 (±2·0)	14·4 (±0·2)	28·1 (±4·2)	4 (±0)

[a] Notation as in Table 2.

[n] Metsulfuron-methyl half-lives were calculated by the adjustment of degradation kinetics to first-order kinetics in the different incubations.

competitive degradation pathway, which could become predominant in acidic environments for metsulfuron-methyl[14,21] and other sulfonylureas.[15,22] Initially the corresponding hydroxy derivative was considered to arise from a biological hydroxylation.[8] However it is now well-known that this kind of product can be produced by chemical reaction without the intervention of micro-organisms.[14,21] The hydroxy-metsulfuron-methyl is less stable than metsulfuron-methyl and the sulfonylurea bridge is slowly hydrolysed, giving the corresponding hydroxy-atrazine (4-hydroxy-6-methyl-2-amino-1,3,5-triazine)[14,21] which could be one of the non-identified metabolites detected. Another degradation pathway of the hydroxy-metsulfuron-methyl is cleavage of the triazine ring, supposed to be an oxidative process with incorporation of three additional atoms of oxygen and loss of an atom of N leading to a stable, less reactive and less water-soluble product.[14,21,24] These complementary degradation pathways have been well identified in laboratory abiotic conditions in the absence of soil, and triazine ring cleavage has been detected at low pH as the major hydrolytic pathway for metsulfuron-methyl.[15] However, during incubations of metsulfuron-methyl in soils, the metabolites arising from

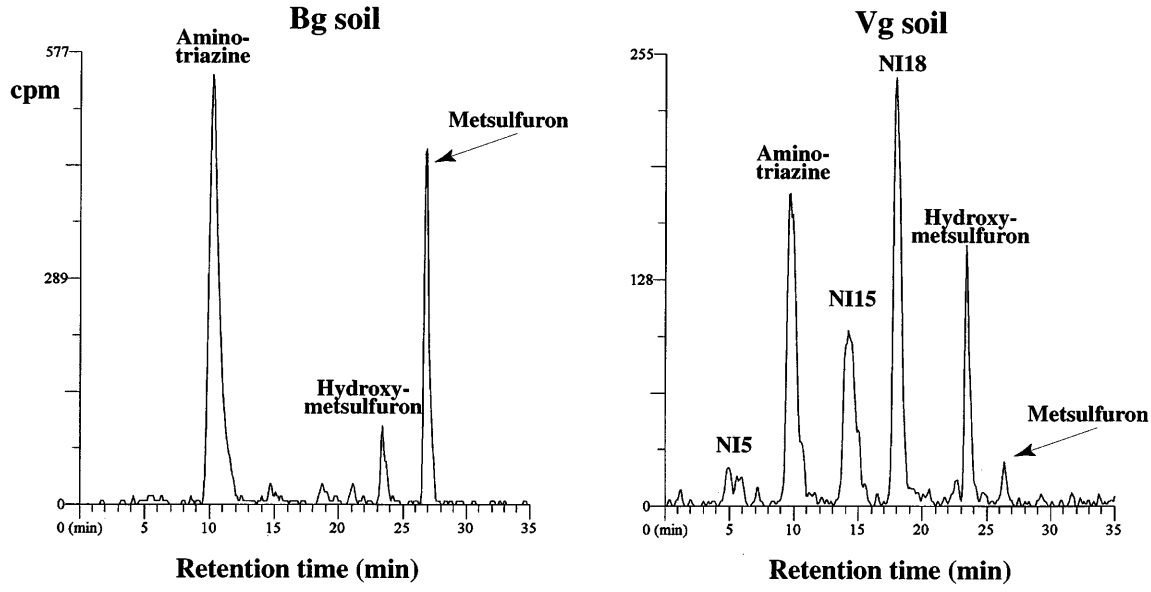

Fig. 2. Examples of HPLC chromatograms with [^{14}C] detection of the water-soluble residues of [^{14}C]metsulfuron-methyl extracted from alkaline Bg and acidic Vg soils after 42 days of incubation at 90% WHC and 28°C.

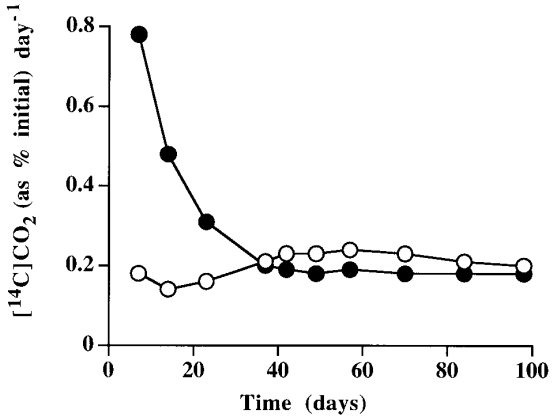

Fig. 3. Rates of [^{14}C]metsulfuron-methyl mineralisation during incubations at 90% WHC and 28°C in (○) alkaline Bg and (●) acidic Vg soils.

hydroxy-metsulfuron-methyl were not detected, although three new non-identified metabolites appeared.[19] This diversity of metabolites containing the [^{14}C]-labelled triazine moiety was concordant with our results; however no supplementary information was found in the literature. The probable biological or abiotic origin of these metabolites will be discussed later with the results of the incubations in sterile conditions.

Bound residues were formed rapidly in both soils and their proportions remained practically constant during the incubations (Fig. 1). The extent of bound residue formation was larger in the acidic Vg soil than in Bg soil: respectively 27 and 14% of the applied [^{14}C]metsulfuron-methyl at the beginning of the incubation. The rates of bound residue formation were comparable to those given in the literature for metsulfuron-methyl.[19,20]

The mineralisation of the [^{14}C]-labelled triazine part of the [^{14}C]metsulfuron-methyl was lower in the Bg soil than in the Vg soil. After 98 days of incubation, the [^{14}C]carbon dioxide evolved represented 20% of [^{14}C]metsulfuron-methyl applied on Bg soil incubated at 28°C and 90% WHC, whereas it was 25% in Vg soil for the same conditions (Fig. 1, Table 3). The metsulfuron-methyl mineralisation in Bg soil increased linearly with time, with a constant mineralisation rate of 0·200 (±0·003)% of the initial [^{14}C] mineralised per day of incubation. In Vg soil, the mineralisation rate was higher than in Bg at the beginning of the incubation, then decreased after 40 days to values similar to those in Bg (Fig. 3). The greater mineralisation in the acidic Vg soil than in the alkaline Bg soil was accompanied by a wider variety of metabolites in the Vg soil.

3.2 Metsulfuron-methyl degradation in sterile conditions

The distribution of radioactivity from [^{14}C]metsulfuron-methyl incubated in Bg and Vg soils at 28°C and 90 WHC in sterile conditions is shown in Fig. 4 and examples of corresponding metabolic profiles are in Fig. 5. Effective soil sterilisation is hard to obtain. Here, drastic autoclaving allowed an efficacious sterilisation of both soils, without any significant modification of the soil pH. The quality of the sterilisation was indicated by the absence of micro-organisms in the sterilised soil samples up to 42 days of incubation (control tests were run by classical enumeration techniques). At the end of the incubation some bacteria were detected. The first consequence, common to both soils, was the

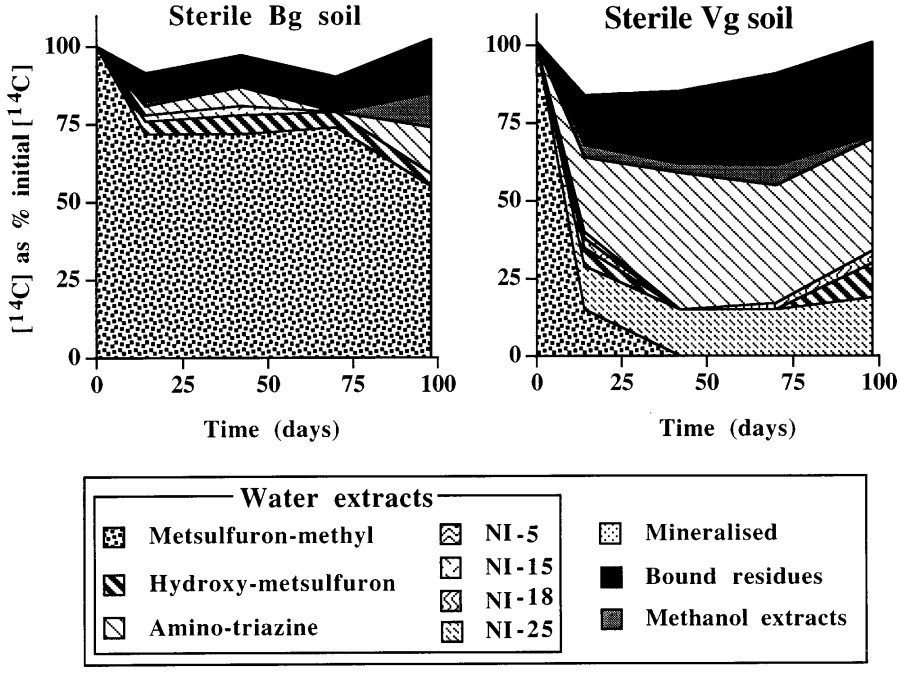

Fig. 4. Fate of [^{14}C]metsulfuron-methyl during incubations in sterile conditions at 90% WHC and 28°C in alkaline Bg and acidic Vg soils.

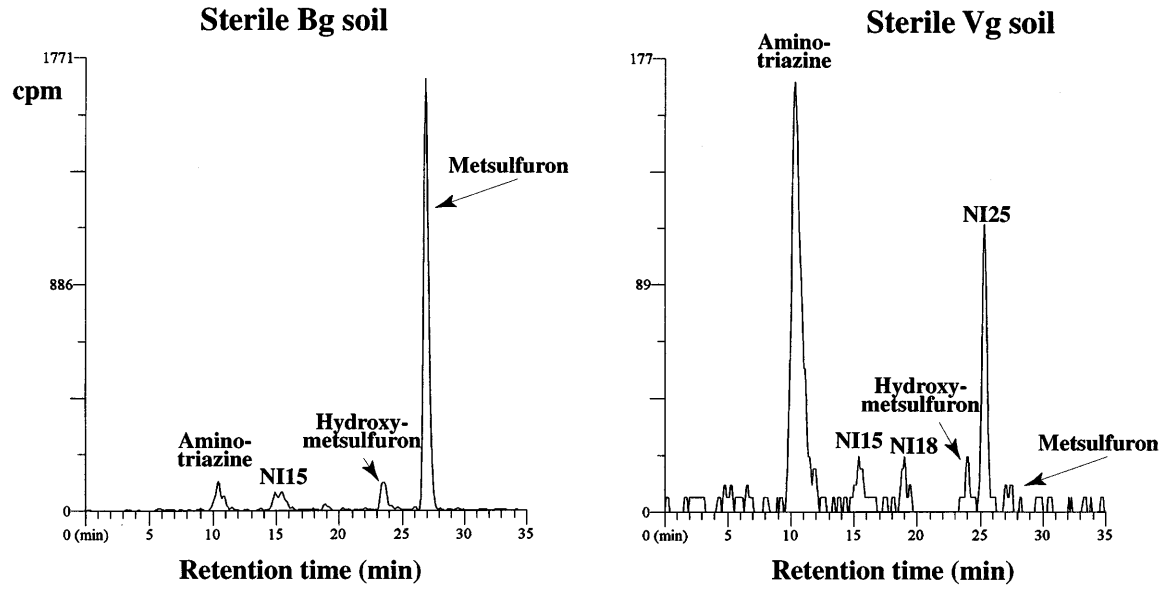

Fig. 5. Examples of HPLC chromatograms with [^{14}C] detection of the water-soluble residues of the [^{14}C]metsulfuron-methyl extracted from alkaline Bg and acidic Vg soils after 42 days of incubation at 90% WHC and 28°C in sterile conditions.

absence of metsulfuron-methyl mineralisation during incubation.

In sterile conditions, only slow degradation of [^{14}C]metsulfuron-methyl was found in Bg soil. After 70 days of incubation, 74% of the initial metsulfuron-methyl remained in the soil, with only 12% corresponding to degradation products (amino-triazine and hydroxy-metsulfuron-methyl). Thereafter the proportion of metsulfuron-methyl decreased to 55% due to the increase of amino-triazine and of the methanol extract. Bound residues were rapidly formed and their proportion remained between 10 and 17% of the initial [^{14}C]metsulfuron-methyl throughout the incubation (Table 3).

In contrast to the Bg soil, incubation of acidic Vg soil in sterile conditions did not lead up to a decrease of metsulfuron-methyl degradation (Fig. 4). After 14 days of incubation, only 15% of the initial metsulfuron-methyl remained in the sterile soil, as in the non-sterile soil. After 42 days of incubation, the metsulfuron-methyl had completely disappeared. These results confirmed the importance of abiotic degradation of this compound. The first-order degradation rate in sterile Vg soil was similar to that in non-sterile soil with the same half-life of five days (Table 3). The inhibition of soil microbial activity had different effects on sulfonylurea degradation, which was very dependent on the soil characteristics. Often a decrease of the degradation rate was observed, due to the elimination of the biological degradation process.[12,13,19] However, similar degradation rates in sterile and non-sterile conditions were observed in soils with high degradation capacity,[25] which was the case for the acidic soil studied here.

Appearance of metabolites differed between sterile and non-sterile incubations (Figs 1 and 4). Fourteen days after [^{14}C]metsulfuron-methyl application to sterile Vg soil, the proportion of the amino-triazine increased compared to non-sterilised soil. The formation of this amino-triazine has been attributed in the literature to the abiotic hydrolysis of the metsulfuron-methyl.[8,16,26] Thus, in the acidic soil, non-ionised metsulfuron-methyl could be hydrolysed through the breaking of the sulfonylurea bridge, followed by the degradation of the carbamate produced.[16] Nevertheless, amino-triazine formation did not seem exclusive to abiotic hydrolysis in acidic conditions; its proportion was also high in the alkaline Bg soil in non-sterile conditions, and the reduction of biological activity in this soil drastically reduced amino-triazine production.

Another important modification of the metabolic profiles during sterile incubations of Vg soil was the low formation of the non-identified metabolite NI-18, whereas a new non-identified metabolite (NI-25) appeared (Figs 4 and 5). This last metabolite was also detected at the end of the incubation of Bg soil in non-sterile conditions (Fig. 1).

Oxidative *O*-demethylation of metsulfuron-methyl, leading to the formation of hydroxy-metsulfuron-methyl, occurred in abiotic conditions, as reported in the literature.[14] However, soil micro-organisms are also able to effect this reaction.[8] After this *O*-demethylation, the triazine ring could be opened by an oxidative process forming the stable product mentioned above. The sulfonylurea bridge of the hydroxy-metsulfuron-methyl can also be hydrolysed giving 4-hydroxy-6-methyl-2-amino-1,3,5-triazine and a sulfonamide. This

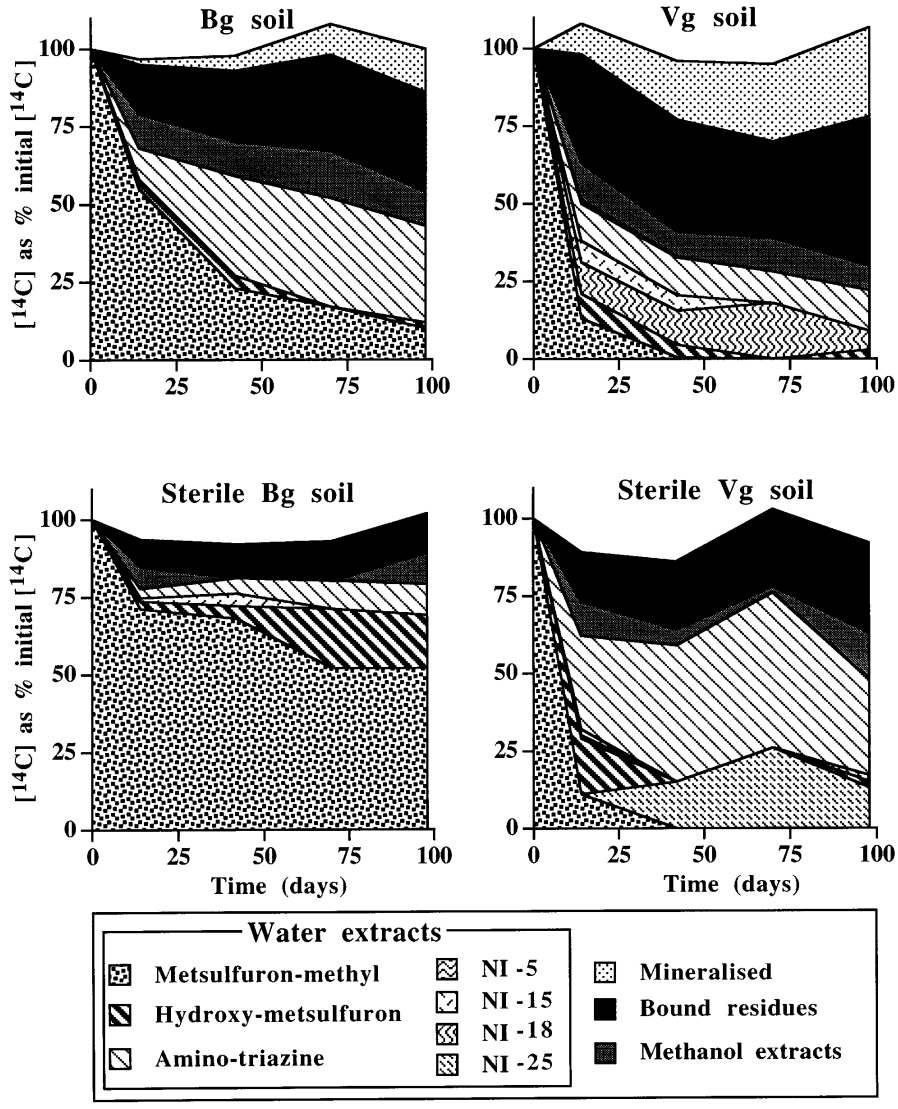

Fig. 6. Fate of [^{14}C]metsulfuron-methyl during incubations in sterile and non-sterile conditions at 50% WHC and 28°C in alkaline Bg and acidic Vg soils.

final degradation process leads to the formation of [^{14}C]carbon dioxide after mineralisation of the triazine, but was not observed in sterile conditions.

3.3 Effect of soil water content on metsulfuron-methyl behaviour

Figure 6 shows the evolution of the radioactive residues from [^{14}C]metsulfuron-methyl during incubations in soils at 50% WHC and 28°C under sterile and non-sterile conditions. No important modifications were found when compared to the results obtained on non-sterile soils at 90% WHC (Fig. 1). In Bg soil, metsulfuron-methyl degradation was slightly decreased, along with a decrease in mineralised metsulfuron-methyl. The half-life was 22 (±2) days against 17 (±2) days found during Bg soil incubation at 90% WHC (Table 3). This could be related to a decrease in biological activity with the change in soil water content. In contrast, in the Vg soil, the half-life did not increase when the soil water content decreased. Within the range of soil water contents studied, this factor seemed to influence only the biological degradation process without any effect on the abiotic degradation process. The increase of the degradation rate with increasing soil water content was interpreted as an increase of the soil microbial activity. However various authors have found that soil water content has a minor effect on the degradation of different sulfonylureas or that the effect depends on the soil type.[3,5,6]

The soil water content did not modify the metabolic profiles in the two soils. The same metabolites were identified at both moisture levels, in the same proportions (Figs 1 and 6). The only difference was the absence from Bg soil at 50% WHC of the metabolite NI-25 detected at the end of incubations at 90% WHC. The proportion of bound residues increased when soil water content decreased in both soils. The increase was higher

in Vg soil than in Bg soil. At the end of the incubation, bound residues represented 48% of the [^{14}C]metsulfuron-methyl applied in Vg soil and 32% in Bg soil (Table 3).

In sterile conditions, the decrease of the soil water content did not modify the evolution of the radioactivity distribution in either soil (Figs 4 and 6). Incubations in sterile conditions at different soil water contents were done to test the influence of the increase of water hydrolytic activity when water content decreased. Indeed, the lower the soil water content, the higher the water activity due to polarity induced by the charged soil surfaces. The only significant difference was the formation of a high proportion of hydroxy-metsulfuron when the sterile soils were incubated at 50% WHC. In the Vg soil, hydroxy-metsulfuron was detected mainly at the beginning of the incubation at 50% WHC and represented 18% of the initial metsulfuron-methyl. On the other hand, in the Bg soil, the hydroxy-metsulfuron was formed mainly at the end of the incubation representing 17 to 19% of the initial metsulfuron-methyl (Fig. 6). The formation of this hydroxylated derivative could be due to enhancement of the water hydrolytic activity. A high proportion of hydroxy-metsulfuron-methyl has been found when degradation occurs on the acidic surfaces of montmorillonite or humic acids. In these conditions, *O*-demethylation and then triazine ring cleavage are favoured.[21,22] Nevertheless, this higher activity did not have a significant influence on the hydrolysis of the sulfonylurea bridge.

3.4 Effect soil temperature

Figure 7 shows the evolution of [^{14}C]metsulfuron-methyl incubated in the two soils at 50 and 90% WHC and 10°C. Examples of chromatograms obtained from samples incubated for 42 days at 10°C and 90% WHC are given in Fig. 8. Decrease in temperature induced an increase in metsulfuron-methyl persistence in both soils

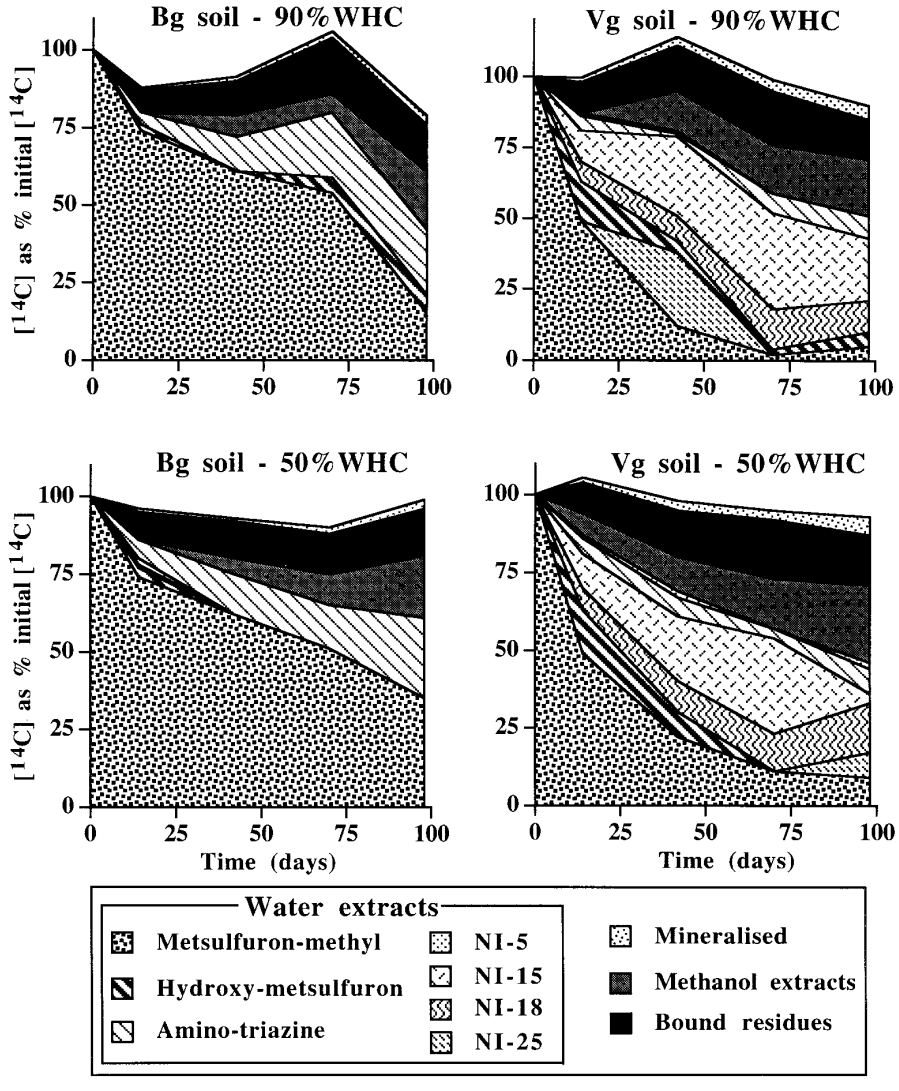

Fig. 7. Fate of [^{14}C]metsulfuron-methyl during incubations at 90 and 50% WHC and 10°C in alkaline Bg and acidic Vg soils.

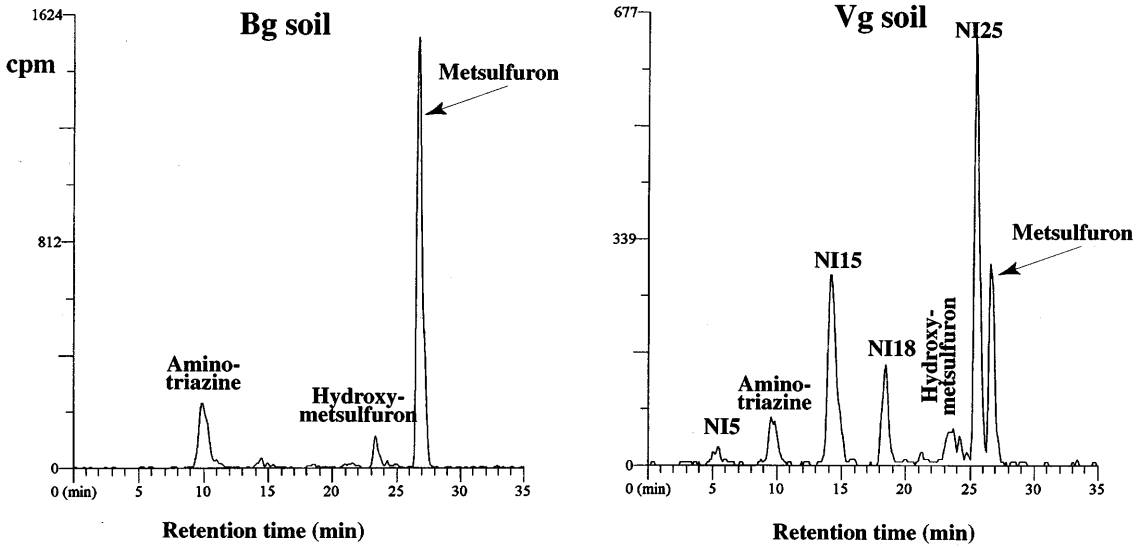

Fig. 8. Examples of HPLC chromatograms with [^{14}C] detection of the water-soluble residues of [^{14}C]metsulfuron-methyl extracted from alkaline Bg and acidic Vg soils after 42 days of incubation at 90% WHC and 10°C.

(Figs 1, 6 and 7). At 10°C, the half-life of metsulfuron-methyl was 53 (±10) days in the alkaline Bg soil (compared to 17 days at 28°C), and 17 (±3) days in the acidic Vg soil (compared to five days at 28°C) (Table 3). These were the only experimental conditions allowing the conservation of some metsulfuron-methyl (5% of the initial amount) at the end of the incubation in Vg soil. The temperature decrease seemed to affect biological degradation process more extensively than the chemical process. This was corroborated by the drastic decrease of metsulfuron-methyl mineralisation in both soils: only 3 and 5% of the initial metsulfuron-methyl were mineralised at the end of the incubation in Bg and Vg soils respectively (Table 3). The temperature dependency of metsulfuron-methyl degradation has been reported previously.[19] Thus, James *et al.*[10] showed that an increase of temperature from 10 to 30°C, decreased the half-life of metsulfuron-methyl by 75% which corresponds to the decrease in half-life observed in this work.

In Bg soil, the same metabolites were identified at 10 and 28°C (Figs 2 and 8) but in different proportions. The proportion of amino-triazine decreased at 10°C. Increase of temperature favours the hydrolysis of the sulfonylurea bridge of metsulfuron-methyl[14] and of other sulfonylureas.[27] In contrast, the temperature decrease markedly modified the metabolic profile of metsulfuron-methyl degradation in the acidic Vg soil (Fig. 8). The proportions of the major metabolites found at 28°C (amino-triazine and NI-18) (Fig. 2) decreased. At 10°C the proportion of amino-triazine was low throughout, with a maximum of only 8% of the initial metsulfuron-methyl at the end of incubation. The metabolic profile was mainly characterised by the early formation of the non-identified metabolite NI-25, which could represent up to 34% of the initial metsulfuron-methyl after 42 days of incubation. This metabolite was also found in this same soil when the incubations were done in sterile conditions, and its formation in this acidic soil could be related to a decrease of the biological activity due to sterile conditions or to low temperature.

The influence of temperature on the degradation of sulfonylurea can be described by an Arrhenius equation:

$$k = A\, e^{-Ea/RT}$$

where k is the first order degradation rate constant (day^{-1}) at temperature T, A is a constant related to the non-thermal factors, T is the absolute temperature (K), R the gas constant (8·314 J K^{-1} mol^{-1}) and Ea the energy of activation (J mol^{-1}). The energy of activation was calculated from the following transformed equation with the data obtained from the incubations at 10 and 28°C:

$$Ea = R \frac{T_{10}\, T_{28}}{T_{28} - T_{10}} \ln \frac{k_{28}}{k_{10}}$$

(k_{10} and k_{28} are the degradation rates of 10°C (T_{10}) and 28°C (T_{28}). No significant difference was found between the values of Ea calculated for metsulfuron-methyl degradation in the two soils; 46 (±4) kJ mol^{-1} and 48 (±4) kJ mol^{-1} for Bg and Vg soils, respectively. No significant difference was found between the values of Ea calculated at the two different soil water contents. Ea values in soils for different sulfonylureas reported in the literature vary from 20 to 130 kJ mol^{-1}.[4,8,28] Usually, abiotic degradation results in higher Ea values than biological degradation. Reported Ea values of acid-catalysed hydrolysis of sulfonylureas have usually ranged between 90 and 140 kJ mol^{-1},[8,15] although

lower *E*a values (30 to 75 kJ mol^{-1}) have been reported for the chemical hydrolysis of some sulfonylureas.[29] One value found in the literature for the *E*a of the chemical hydrolysis of metsulfuron-methyl was 84 kJ mol^{-1}.[14] However, in the present work, no significant thermodynamic difference was found between metsulfuron-methyl degradation in the two soils. If the degradation really occurred mainly through abiotic mechanisms in the acidic soil, this did not need supplementary energy as compared to biological degradation in the other soil. On the other hand, the relative low *E*a value did not reflect any chemical mechanism limiting the rate of degradation.

The evolution of the radioactive distribution in soil samples incubated at 50% WHC at different temperatures confirmed the greater influence of temperature than of soil water content (Figs 6 and 7). The radioactive distributions in Bg and Vg soils were similar for the same temperature at 50 and 90% WHC (Fig. 7). At 50% WHC and 10°C, the degradation of metsulfuron-methyl was low in the Bg soil and the main metabolite was amino-triazine, which represented 26% of the applied metsulfuron-methyl at the end of the incubation. In the Vg soil, the half-life of metsulfuron was 17 days, as during the incubation at 90% WHC at the same temperature (Table 3). The metabolic profile was also similar and always characterised by the presence of the specific NI-15 metabolite. The only qualitative difference in the metabolite distribution in Vg soil between 50 and 90% WHC at 10°C was the point where the metabolite NI-25 was first detected during the incubation: at the end of the incubation at 50% WHC and between 14 and 70 days at 90% WHC.

4 CONCLUSIONS

Figure 9 summarises the results obtained concerning metsulfuron-methyl behaviour in the two soils. A Principal Component Analysis was conducted with the following variables: soil pH, temperature and distribution of the radioactivity from [^{14}C]metsulfuron-methyl, including the metabolites, in the different analysed compartments. The observations corresponded to all the times (14, 42, 70 and 98 days) in all the conditions of incubation. The different incubations have been identi-

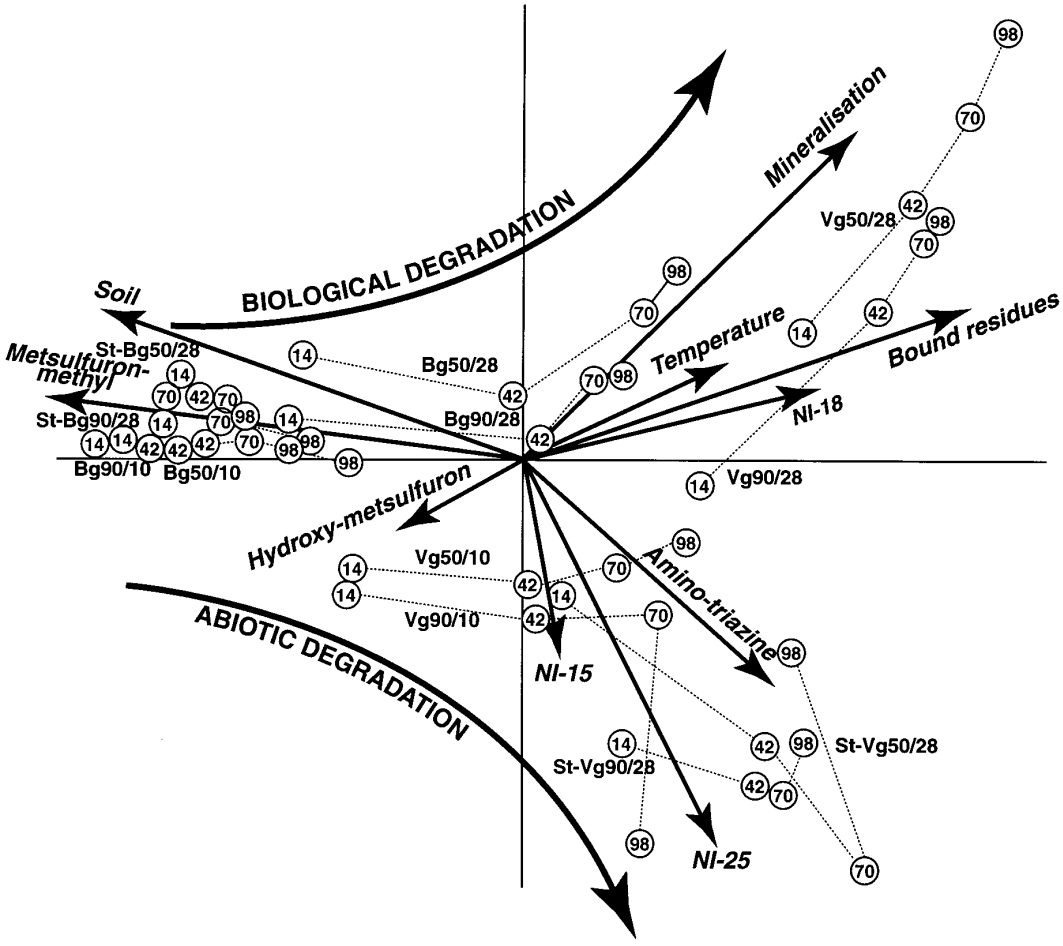

Fig. 9. Result of a Principal Component Analysis realised with all data from all incubation conditions and with the following variables: soil pH, temperature and distribution of the radioactivity, including the detected metabolites, in the different analysed compartments.

fied in Fig. 9 following the notation used in Table 2, and the points corresponding to the different dates for the same incubation have been linked. The horizontal axis in the graph corresponded to the principal component, which accounted for 30% of the total variation. This axis corresponded well to metsulfuron-methyl degradation, which increased from left to right. Thus, soil samples with low metsulfuron-methyl degradation (alkaline soil in sterile conditions or low temperature) formed a cloud of points on the left end of the axis. The vertical axis in Fig. 9 represented only 13% of the total variation but allowed a good discrimination between samples in which degradation was mainly abiotic (acidic soil in sterile conditions or low temperature) and samples with both biological and abiotic degradations. The representation of the vectors corresponding to variables in the same plan allowed the following conclusions: (1) metsulfuron-methyl degradation was strongly correlated to soil pH; in agreement with the literature, increase of soil pH induced an increase of metsulfuron persistence; (2) bound residues formation and metsulfuron-methyl mineralisation were correlated; both were inversely correlated to metsulfuron-methyl degradation and soil pH; (3) metsulfuron-methyl degradation was mainly correlated with the production of the amino-triazine, and of the non-identified metabolites NI-25 and NI-18; (4) abiotic degradation of the metsulfuron-methyl was characterised mainly by the formation of the amino-triazine and the metabolite NI-25.

In acidic soil, degradation of the metsulfuron-methyl was higher than in alkaline soil, due to the combined actions of chemical hydrolysis and micro-organisms. In these conditions, the degradation rate was higher than in the alkaline soil, and the formation of specific metabolites (NI-15 and NI-18) occurred; these were non-detected or detected at only a low level during incubation in the alkaline soil. The degradation of metsulfuron-methyl was very dependent on temperature, both for the biological and the abiotic processes. On the other hand, the influence of the soil water content on degradation seemed very low in the range of water contents tested.

All the results shown were obtained with a high applied amount of metsulfuron-methyl (23 $\mu g\ kg^{-1}$), corresponding to about 15 times the usual agronomic amount. That was necessary because of the low specific activity of the [^{14}C]metsulfuron-methyl used, and large amounts were necessary to provide good detection limits. Control incubations (results not shown) using agronomic doses (1·5 $\mu g\ kg^{-1}$) at 28°C and 90% WHC gave similar patterns of [^{14}C] distribution between the mineralised fraction, the water- and methanol-extractable fractions and the bound residues at the two concentrations. The lack of influence of concentration was not verified for the other incubation conditions, and the effect of concentration on the formation of metabolites from the metsulfuron-methyl should be investigated.

ACKNOWLEDGEMENTS

This work was done in collaboration between INRA and ITCF (Boigneville, France). Thanks are due to French Agriculture Minister for financial support, to Du Pont de Nemours for supplying radiolabelled metsulfuron-methyl, and to Dr J. Bastide (GERAP, Perpignan, France) for supplying metsulfuron-methyl metabolites.

REFERENCES

1. Beyer, E. M., Brown, H. M. & Duffy, M. J., Sulfonylurea herbicide soil relation. *Proc. Brighton Crop Protect. Conf.—Weeds*, 1987, 531–40.
2. Brown, H. M., Mode of action, crop selectivity and soil relations of the sulfonylurea herbicides. *Pestic. Sci.*, **29** (1990) 263–81.
3. Anderson, R. L., Environmental effects on metsulfuron and chlorsulfuron bioactivity in soil. *J. Environ. Qual.*, **14** (1985) 517–21.
4. Thirunarayanan, K., Zimdahl, R. L. & Smika, D. E., Chlorsulfuron adsorption and degradation in soil. *Weed Sci.*, **33** (1985) 558–63.
5. Fuesler, T. P. & Hanafey, M. K., Effect of moisture on chlorimuron degradation in soil. *Weed Sci.*, **38** (1990) 256–61.
6. Oppong, F. K. & Sagar, G. R., Degradation of triasulfuron in soil under laboratory conditions. *Weed Res.*, **32** (1992) 167–73.
7. Fredrickson, D. R. & Shea, P. J., Effect of pH on degradation, movement and plant uptake of chlorsulfuron. *Weed Sci.*, **34** (1986) 328–32.
8. Beyer, E. M., Duffy, M. J., Hay, J. U. & Schlueter, D. D., Sulfonylureas herbicides. In *Herbicides: Chemistry, Degradation and Mode of Action*, ed. P. C. Kearney & D. D. Kaufman. Marcel Dekker, New York, 1987, pp. 117–89.
9. Walker, A., Cotteril, E. G. & Welch, S. J., Adsorption and degradation of chlorsulfuron and metsulfuron-methyl in soils from different depths. *Weed Res.*, **29** (1989) 281–7.
10. James, T. K., Klaffenbach, P., Holland, P. T. & Rahman, A., Degradation of primisulfuron-methyl and metsulfuron-methyl in soil. *Weed Res.*, **35** (1995) 113–20.
11. Walker, A. & Welch, S. J., The relative movement and persistence in soil of chlorsulfuron, metsulfuron-methyl and triasulfuron. *Weed Res.*, **29** (1989) 375–83.
12. Ismail, B. S. & Lee, H. J., Persistence of metsulfuron-methyl in two soils. *J. Environ. Sci. Health.*, **B30** (1995) 485–97.
13. Joshi, M. M., Brown, H. M. & Romesser, J. A., Degradation of chlorsulfuron by soil microorganisms. *Weed Sci.*, **33** (1985) 888–93.
14. Sabadie, J., Hydrolyse chimique acide du metsulfuron-méthyle. *Weed Res.*, **30** (1990) 413–19.
15. Berger, B. M. & Wolfe, N. L., Hydrolysis and biodegradation of sulfonylurea herbicides in aqueous buffers and anaerobic water-sediment systems: assessing fate pathways using molecular descriptors. *Environ. Toxicol. Chem.*, **9** (1996) 1500–7.

16. Hemmamda, S., Calmon, M. & Calmon, J. P., Kinetics and hydrolysis mechanism of chlorsulfuron and metsulfuron-methyl. *Pestic. Sci.*, **40** (1994) 71–6.
17. Nicholls, P. H. & Evans, A. A., The behaviour of chlorsulfuron and metsulfuron in soils in relation to incidents of injury to sugarbeet. *Proc. Brighton Crop Protect. Conf.—Weeds*, 1987, 549–56.
18. Kotoula-Syka, E., Eleftherohorinos, I. G., Gagianas, A. A. & Sficas, A. G., Phytotoxicity and persistence of chlorsulfuron, metsulfuron-methyl, triasulfuron and tribenuron-methyl in three soils. *Weed Res.*, **33** (1993) 355–67.
19. Vega, D., Bastide, J. & Poulain, C., Dégradation chimique ou microbiologique des sulfonylurées dans le sol. II. Cas du metsulfuron-méthyle. *Weed Res.*, **32** (1992) 149–55.
20. Smith, A. E., Persistence of the herbicides [^{14}C]chlorsulfuron and [^{14}C]metsulfuron-methyl in Prairies soils under laboratory conditions. *Bull. Environ. Contam. Toxicol.*, **37** (1986) 698–704.
21. Sabadie, J. & Bastide, J., Dégradation du metsulfuron-méthyle déposé sur divers supports minéraux. *Weed Res.*, **30** (1990) 1–8.
22. Sabadie, J., Dégradation du chlorsulfuron et du metsulfuron-méthyle en présence d'acides humiques. *Weed Res.*, **33** (1993) 397–407.
23. Badon, R., Bastide, J. & Sabadie, J., Réactivité de l'herbicide metsulfuron-méthyle, synthèse des produits de dégradation. *Chemosphere*, **21** (1990) 289–94.
24. Sabadie, J., Réactivité de l'herbicide chlorsulfuron; synthèse et structure de ses produits de dégradation. *Weed Res.*, **32** (1992) 137–42.
25. Anderson, J. J. & Dulka, J. J., Environmental fate of sulfometuron-methyl in aerobic soils. *J. Agric. Food Chem.*, **33** (1985) 596–602.
26. Harvey, Dulka, J. J. & Anderson, J. J., Properties of sulfometuron-methyl affecting its environmental fate: aqueous hydrolysis and photolysis, mobility and adsorption on soils, and bioaccumulation potential. *J. Agric. Food Chem.*, **33** (1985) 590–6.
27. Long, A. R., Charkhian, B., Hsieh, L. C., Short, C. R. & Barker, S. A., Characterization of the thermal decomposition products of the sulfonylurea herbicide chlorsulfuron. *J. Chrom.*, **505** (1990) 395–401.
28. Ravelli, A., Pantani, O., Calami, L. & Fusi, P., Rates of chlorsulfuron degradation in three Brazilian oxisols. *Weed Res.*, **37** (1997) 51–9.
29. Dinelli, G., Vicari, A., Bonetti, A. & Catizone, P., Hydrolytic dissipation of four sulfonylurea herbicides. *J. Agric. Food Chem.*, **45** (1997) 1940–5.